Disclosure Statement on June 24, 2003) not reflecting the protein quantities prove that this is not a common knowledge of the one of ordinary skill in the art.

3. On pages 2-3 of the response mailed on February 6, 2007, the applicant explained that "BSA is commonly used in protein quantity estimation, so do other proteins such as lysozyme, IgG, and insulin. Any known protein with know quantity may be used as a protein standard." There are thousands of known proteins. If one of these known proteins is used as standard in estimating the quantity of conalbumin and another of these known protein used as standard in estimating the quantity of ovalbumin, the staining intensities of resulting conalbumin and ovalbumin will not reflect their quantities. This is what the applicant was trying to explain on pages 2-3 of the response mailed on February 6, 2007.

4. Many proteins may be used as a protein standard in protein quantitation. Many different assays may be used in protein quantity estimation. Some of the protein quantitation assays may not need a protein standard. For example, UV absorbance can estimate protein quantity by detecting its aromatic amino acid composition without a protein standard. In the case that one protein is used as a standard to estimate the quantity of proteins by different assays, these estimated proteins cannot be used as protein standard to estimate quantity of a sample protein since their detection intensities do not reflect their quantities.

The most commonly used protein assays to estimate quantity of proteins are BCA assay, Bradford assay, UV absorbance and weighing by an analytical balance. These procedures can be completed in minutes. Electrophoresis on a gel followed by staining with Coomassie Blue, silver stain, or any other stains will require hours of operation and hours or overnight destain. Conalbumin, ovalbumin, and lysozyme used by Mizutani were most likely assayed by BCA, Bradford, or UV absorbance. If these proteins were obtained from vendors in powder forms, their quantities were most likely obtained by weighing. Proteins prepared by these assays cannot be used as quantitative protein standard since their staining intensities do not reflect their quantities. Mizutani did not teach the claimed protein standard. Mizutani did not teach the claimed method to make the claimed standard. Although the proteins used by Mizutani and used in Example 1 of the application are same, but they have intrinsically different properties namely if their staining intensities reflect their quantities. On the other hand, proteins used in Example 1

and Example 2 of the application are completely different, but their staining intensities reflect their quantities after electrophoresis on a gel followed by Coomassie Blue staining. Therefore these proteins have intrinsically similar properties. The properties of these proteins are obtained only by extensive testing, experimentation, and introduction of inventive steps. Mizutani's teaching, other prior arts or combination of them did not teach the claimed invention.

Only after extensive testing, experimentation, and inventive steps are introduced, the claimed invention demonstrated that (1) Different detection assays cannot be used in the protein quantity determination for the polypeptides in one protein standard. If different assays use one known protein as standard protein to estimate the quantities of different polypeptides in the quantitative protein standard, the resulting quantitative protein standard can only be used as a size standard. (2) Protein quantities determined by one detection assay are reproducible only if same procedure is used in determining quantity of each polypeptide in the quantitative protein standard and in using the quantitative protein standard. If Coomassie Blue staining is used in using the quantitative protein standard on a gel, the quantitative protein standard has to be made with the Coomassie Blue staining on a gel and vice versa. (3) Protein quantities determined by one assay with same protein such as BSA as a standard are not same in solution and on a gel. Coomassie Blue staining can be used both in solution and on gel protein staining. From extensive experiments, the applicant reasoned that gel electrophoresis, staining and destaining procedures eliminate the effects of different buffers, salts, detergents, reducing agents and impurities in different protein samples. Therefore on gel staining results are more accurate than in solution staining in protein amount estimation. This is why Coomassie Blue staining on a gel is used in protein quantity estimation in making the quantitative protein standard for each of the polypeptides in all examples of the disclosed invention. On gel staining is not only used in using the protein standard, it is also used in making the protein standard. (4) Staining and destaining variations such as time, solution concentration, and temperature affect reproducibility. The quantities of all proteins used in a protein standard were estimated and should be estimated simultaneously on a gel in one experiment. All these are inventive steps in the claimed invention. Involvement of extensive testing, experiments and introduction of multiple inventive steps in making and

using the quantitative protein standard indicate it is not obvious to one of ordinary skill in the art at the time of the instant invention to make the quantitative protein standard. Therefore proteins taught by Mizutani cannot be used as standard to determine the quantity of a sample protein on a gel since their quantities were not determined by the method of making the claimed standard disclosed in the application. Mizutani did not teach how to estimating quantity of a protein which the staining intensity will reflect its quantity. The claimed protein standard, the methods of making and using the standard disclosed in the application are not obvious over Mizutani's teaching or other prior arts or combination of them.

5. The fact that relatively large biotech reagent companies such as Invitrogen, Fisher Scientific, and Novagen have not developed a protein standard which can estimate size and quantity of a sample protein. The fact that Mizutani's patent was granted on April 29, 1975 which is over 30 years ago. The fact that scientists from academic labs and industries purchased our quantitative protein standard at three times of the price of regular protein size standards of companies such as Invitrogen, Fisher Scientific, and Novagen. All these facts clearly indicate that Mizutani did not teach a quantitative protein stand that can be used to estimate protein size and quantity on a gel. These facts also indicate that it is not obvious to make the claimed standard over Mizutani's art. In addition, these facts indicate the claimed protein standard solves a long-felt, long existing but unsolved need.

6. The claimed protein standard, the methods of making and using the standard are integral parts of the claimed invention and they are invented simultaneously. Without the claimed protein standard and method of making it, the method of using is not going to work on other protein standards. For example, the proteins taught by Mizutani or other protein standards by Fisher Scientific or Novagen cannot be used as a standard to determine protein quantity on a gel even though the method of using disclosed in the application is used since the staining intensities will not reflect the protein amounts. Without the claimed quantitative protein standard and method of making it, the method of using the standard would not have been developed. Claims 39-55 are cancelled. New claims 57 to 67 are presented to reflect the integrity of the claimed invention.

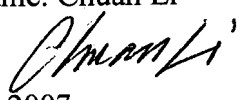
In conclusion, the disclosed invention involves extensive testing, experimentation, and introduction of inventive steps. It is not obvious over the proteins taught by Mizutani, over other prior arts, or the combination of them. Therefore the new claims are submitted that patentable subject matter is clearly presented. If the examiner agrees but does not feel that the present claims are technically adequate, applicant respectfully requests that the examiner write acceptable claims pursuant to MPEP 707.07(j).

d.) Remarks

Any inquiry concerning these amendments or earlier communications from the applicant should be directed to Chuan Li whose telephone number is (858) 361-7231. The applicant can normally be reached from 9:00 to 5:00 pacific standard time.

The applicant may also be reached at Expression Technologies Inc. at (858) 558-1861 or by fax at (858) 558-1883 or by email at chuanli@exptec.com.

Applicant Name: Chuan Li

Signature: 

Date: June 5, 2007